The incidence of *Borrelia burgdorferi*, *Anaplasma phagocytophilum* and *Babesia microti* coinfections among foresters and farmers in eastern Poland

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ABSTRACT

*Background & objectives:* Lyme borreliosis is the most common tick-borne disease in Europe and the USA. However, a great variety of pathogens are transmitted by ticks, which results in mixed infections, with Lyme borreliosis. The aim of the present study was to show the incidence of *Borrelia burgdorferi*, *Anaplasma phagocytophilum*, and *Babesia microti* coinfections among the population of foresters and farmers, as these people, due to their profession, are particularly exposed to tick contact.

*Methods:* The study was carried out in eastern Poland (the northern part of the Lublin Province) in 2013. The study was performed in a group of 93 individuals occupationally exposed to tick bites (foresters and farmers), whose blood serum showed the presence of IgG anti-*B. burgdorferi* antibodies. Blood serum in this group were evaluated for IgG anti-*A. phagocytophilum* and IgG anti-*B. microti* antibodies by means of IFA IgG indirect immunofluorescence tests. Information related to age, sex, number of tick bite episodes, presence of various symptoms related to the tick bites, and antibiotic therapy applied as treatment for diagnosed Lyme borreliosis were obtained from the subjects through a structured questionnaire. The results were analyzed in Statistica v. 7.1 statistical analysis software.

*Results:* The presence of IgG antibodies against the analyzed pathogens revealed *B. burgdorferi* and *A. phagocytophilum* coinfection in 26 (28%) of the examined subjects and *B. burgdorferi* and *B. microti* coinfection in one person (1.1%). No coinfection with all the three pathogens was observed in any individual. The co-occurrence of headache plus bone, joint and muscle pain was noted significantly more often among individuals diagnosed with *B. burgdorferi* and *A. phagocytophilum* coinfection.

*Interpretation & conclusion:* Foresters and farmers are exposed to *B. burgdorferi* and *A. phagocytophilum* coinfection in the study area. Therefore, it is probable that these pathogens may severely interfere with the clinical course of Lyme borreliosis.

Key words *Anaplasma phagocytophilum; Babesia microti; Borrelia burgdorferi; coinfection, eastern Poland*

INTRODUCTION

Lyme borreliosis (LB) is considered as the most common tick-borne disease in Europe and the USA. However, a great variety of pathogens are transmitted by ticks, which results in mixed infections with Lyme borreliosis¹². *Anaplasma phagocytophilum* bacteria and *Babesia* protozoans are among many pathogens transmitted by ticks.

*Anaplasma phagocytophilum* bacteria (formerly *Ehrlichia phagocytophilum*) invade neutrophilic granulocytes and are responsible for human granulocytic anaplasmosis (HGA). They used to be regarded as etiological agents of infections affecting only animals³. The first case of anaplasmosis in Poland was reported in 2001. It was a mixed infection. The patient was first diagnosed with Lyme borreliosis, but later some non-specific hematological symptoms developed⁴. Currently, a few cases with fully developed anaplasmosis symptoms are detected every year³. In the years of 2006–12, in Laboratory of Rickettsiae, Chlamydiae, and Spirochetes, National Institute of Public Health–National Institute of Hygiene (NIPH-NIH), Warsaw, Poland, this disease was observed in nine patients coming from different parts of Poland⁵. The course and intensity of *A. phagocytophilum* may take different forms ranging from asymptomatic to very serious cases¹³. The clinical symptoms, which are not very distinctive, may hinder the identification of anaplasmosis and its differentiation from other diseases by practitioners. The most common symptoms are those typical of flu, *i.e.* high fever, muscle and joint pains, weakness, headache, dizziness, vomiting, diarrhoea as well as hepatomegaly and splenomegaly. Some patients may suffer from pneumonia and renal insufficiency or neurologi-
cal symptoms including consciousness disorders\textsuperscript{2–3}. Treatment is more likely to be effective if started early in the course of the disease. Doxycycline is the first line of treatment and should be initiated immediately whenever anaplasmosis is suspected\textsuperscript{6}.

Another tick-borne pathogen is the Babesia protozoan. It comprises $>110$ species of obligatory intracellular parasites of vertebrates (mammals, birds), including humans\textsuperscript{7}. Their incubation time ranges from 1 to 6 wk after the tick bite. The disease can have either an acute or a mild form or, in some cases, can even be asymptomatic. Its clinical signs are reminiscent of those in malaria, i.e. headache, muscle and joint pain, nausea, vomiting, high temperature, sweating, shivering, and personality disorders. The maximum number of cases is observed in the US, where the disease is mainly caused by B. microti, while in Europe causative agents are B. divergens and B. bovis\textsuperscript{1, 8–9}. The presence of B. microti on the European continent has been confirmed in both the reservoir and Babesia vectors, i.e. Ixodes ricinus ticks\textsuperscript{7}. Serological investigations confirming the presence of antibodies against B. microti in humans\textsuperscript{7, 10–13} suggest that this pathogen triggers the disease in Europe as well. In Poland, only one case of B. microti infection has been reported so far. It is assumed to have been brought from Brazil\textsuperscript{14}.

People working in the areas inhabited by ticks, i.e. foresters clearing and planting new forests, drivers transporting timber, and farmers, are particularly exposed to tick-borne diseases including Lyme borreliosis, anaplasmosis, and babesiosis.

The aim of the study was to demonstrate the prevalence of B. burgdorferi, A. phagocytophilum, and B. microti coinfections among foresters and farmers from the northern part of the Lublin Province, who are occupationally exposed to tick bites and are at high risk of acquiring tick-borne infections.

**MATERIAL & METHODS**

The study was carried out in the northern part of the Lublin Province, in eastern Poland in 2013 (Fig. 1). Agriculture is the major economic activity in this area and forestry plays an important role as well.

The investigations involved 93 individuals occupationally exposed to tick bites (foresters and farmers), whose blood serum showed the presence of IgG anti-B. burgdorferi antibodies. The study group (93 individuals) was selected from 275 workers (foresters and farmers), who were subjected to a routine, two-stage serological diagnostic procedure for Lyme borreliosis. The first stage involved determination of anti-B. burgdorferi sensu lato IgG and IgM antibodies with ELISA method (anti-Borrelia ELISA IgM and anti-Borrelia plus VlsE ELISA IgG, Euroimmun, Germany). The wells were coated with mixed antigens of B. burgdorferi sensu stricto, B. afzelii, B. garinii and recombinant protein VlsE. In accordance with the manufacturer’s recommendations, a result above 22 RU/ml was considered positive, whereas a result within 16–22 RU/ml was regarded as doubtful. The positive and doubtful results obtained were confirmed using the Western blot method (anti-Borrelia EUROLINE-WB IgM/ IgG; Euroimmun, Germany). They test stripes comprised immobilized antigens of B. afzelii (p83, p41, p39/BmpA, p31/OspA, p30, p25/OspC, p21, p19 and p17/DbpA) as well as a chip with recombinant antigen VlsE. The results were read based on the quantity and type of bands, using the EuroLinescan software (Euroimmun, Germany). The results were interpreted according to the manufacturers’ instructions.

The 93 subjects whose blood serum showed the presence of IgG anti-B. burgdorferi antibodies were tested for A. phagocytophilum and B. microti coinfections. IgG anti-A. phagocytophilum and IgG anti-B. microti antibodies in blood serum were examined by means of IFA IgG indirect immunofluorescence tests.

IgG anti-A. phagocytophilum antibodies in blood serum were detected with semi-quantitation indirect immunofluorescence assay (A. phagocytophilum IFA IgG antibody kit, Fuller Laboratories, California, USA). Solid-phase antigens containing fixed THP-1 cells were infected with A. phagocytophilum. The patient sera were
diluted to 1:80. A positive reaction was seen as sharply defined green fluorescent inclusions in the cytoplasm of infected cells. The fluorescence intensity of the positive control diluted to 1:640 was considered as the cut-off value.

IgG anti-\textit{B. microti} antibodies in blood serum were detected with the semi-quantitation indirect immunofluorescence assay (\textit{B. microti} IFA IgG antibody kit, Fuller Laboratories, California, USA). The solid-phase antigens containing hamster or mouse erythrocytes were infected with \textit{B. microti}. The patient sera were diluted to 1:64. A positive reaction was seen as sharply defined green fluorescent inclusions within the infected erythrocytes. The fluorescence intensity of the positive control diluted to 1:512 was considered as the cut-off value.

All the tests were carried out following the manufacturer’s instructions.

After obtaining informed consent, the subjects were asked to fill in a questionnaire including data about the age, sex, number of tick bite episodes, presence of erythema migrans and other symptoms related to the tick bites, and antibiotic therapy used as treatment for diagnosed Lyme borreliosis.

The results obtained were subjected to statistical analyses. The Pearson’s $\chi^2$-test was used to verify the hypothesis of the independence of variables. For statistical analysis, the assumed level of relevance was 0.05. The statistical analysis was carried out by means of Statistica v. 7.1 computer software.

Consent for the study was obtained from the Bioethics Committee at the Medical University in Lublin (KE-0254/286/2011 and KE-0254/12/2013).

RESULTS

The test group was selected from 275 workers (foresters and farmers), who were subjected to a routine, two-stage serological diagnostic procedures for Lyme borreliosis. The positive or doubtful results of ELISA screening test were obtained with 173 subjects (62.9%), whereas IgM and/or IgG anti-\textit{Borrelia} antibodies were found in 123 subjects (44.7%) by means of Western blot test\textsuperscript{15}. Western blot test revealed 98 IgG anti-\textit{Borrelia} positive subjects, and 93 individuals who showed significant levels of antibodies were tested for \textit{B. burgdorferi}, \textit{A. phagocytophilum} and \textit{B. microti} coinfections.

The test group (93 individuals, whose blood serum showed the presence of IgG anti-\textit{B. burgdorferi} antibodies) comprised 81.7% of foresters and 18.3% of farmers, aged between 20 and 64 yr (mean age 46±10 yr). In the group, 79.6% subjects were males and 20.4% were females.

The presence of IgG anti-\textit{B. burgdorferi} and anti-\textit{A. phagocytophilum} or anti-\textit{B. microti} antibodies was found in 27 subjects (29%). However, the simultaneous presence of antibodies against all the three pathogens was not observed in any of the patients. IgG anti-\textit{B. burgdorferi} and anti-\textit{A. phagocytophilum} antibodies were detected in 26 patients (28%) (Fig. 2), whereas IgG anti-\textit{B. burgdorferi} and anti-\textit{B. microti} antibodies were found in only one patient (1.1%) (Fig. 3) (Table 1).

Since a \textit{B. burgdorferi} and \textit{B. microti} coinfection was found in only one person, detailed analysis was carried out solely in relation to subjects who were diagnosed with a \textit{B. burgdorferi} and \textit{A. phagocytophilum} coinfection.

This coinfection was more frequently reported in males (31.1%) than females (15.8%) ($\chi^2=1.76; p=0.1852$), and slightly more often in foresters (29%) than farmers.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{fig2.png}
\caption{A positive reaction IgG anti-\textit{A. phagocytophilum}—Green fluorescent inclusions in the cytoplasm of cells.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{fig3.png}
\caption{A positive reaction IgG anti-\textit{B. microti}—Green fluorescent inclusions in the erythrocytes.}
\end{figure}
Table 1. Presence of IgG anti-\textit{A. phagocytophilum} and anti-\textit{B. microti} antibodies in the blood plasma of foresters and farmers diagnosed with the presence of IgG anti-\textit{B. burgdorferi} antibodies

<table>
<thead>
<tr>
<th>IgG antibodies</th>
<th>Anti-Babesia microti</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Anti-\textit{Anaplasma phagocytophilum} Positive</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Negative</td>
<td>1 (1.1)</td>
</tr>
<tr>
<td>Total</td>
<td>1 (1.1)</td>
</tr>
</tbody>
</table>

Figures in parentheses indicate percentages.

(23.5\%) ($\chi^2$=0.20; $p=0.6527$). The coinfection incidence grew with age, which is shown in Table 2.

In the analyzed group, 89 subjects (95.7\%) declared having been bitten by a tick; 30 of them (32.3\%) reported a single tick bite, whereas 59 (63.4\%) reported multiple bites. The \textit{B. burgdorferi} and \textit{A. phagocytophilum} coinfection was found more often in respondents reporting a single tick bite (33.3\%) than those with multiple bites or none (25.0\%) (Table 2).

Erythema migrans was reported by 20 subjects (21.5\%), but a \textit{B. burgdorferi} and \textit{A. phagocytophilum} coinfection was diagnosed more often in those without the rash (Table 2).

It was found that 53 subjects (57\% of the test group) took antibiotics when diagnosed with a Lyme borreliosis. \textit{Borrelia burgdorferi} and \textit{A. phagocytophilum} coinfection was detected more often in individuals who were not administered any antibiotics (32.5\%) (Table 2).

In the questionnaire, the subjects were asked to describe the after-bite symptoms. Most subjects declared bone and joint pain (65 subjects), muscle pain (48), headache (23), and concentration disorder (14). All these symptoms were more often reported by those who contracted a \textit{B. burgdorferi} and \textit{A. phagocytophilum} coinfection, but the differences were not statistically significant. However, it is worth mentioning that the headache incidence in the group where the coinfection had been diagnosed was close to statistical significance ($\chi^2 = 3.65; p = 0.0559$).

The co-occurrence of headache and bone, joint and muscle pain was noted significantly more often among individuals diagnosed with \textit{B. burgdorferi} and \textit{A. phagocytophilum} coinfection ($\chi^2 = 5.21; p = 0.0225$) (Table 3).

### DISCUSSION

Tick-borne diseases represent a growing problem to public health. Lyme borreliosis is the most frequent tick-borne disease in Poland and the number of registered cases is on the increase. The observed increase in the incidence of Lyme disease is multi-faceted and includes the etiological agent, vector, and reservoir of the spirochetes.$^{16}$

### Table 3. \textit{Borrelia burgdorferi} and \textit{A. phagocytophilum} coinfection incidence in relation to the symptoms reported

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Anti-\textit{A. phagocytophilum}</th>
<th>Test value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone and joint pain</td>
<td>Positive</td>
<td>$\chi^2$</td>
</tr>
<tr>
<td>Reported</td>
<td>19 (73.1)</td>
<td>0.17</td>
</tr>
<tr>
<td>Not reported</td>
<td>7 (26.9)</td>
<td>0.6767</td>
</tr>
<tr>
<td>Muscle pain</td>
<td>Positive</td>
<td>$\chi^2$</td>
</tr>
<tr>
<td>Reported</td>
<td>16 (61.5)</td>
<td>1.42</td>
</tr>
<tr>
<td>Not reported</td>
<td>10 (38.5)</td>
<td>0.2328</td>
</tr>
<tr>
<td>Headache</td>
<td>Positive</td>
<td>$\chi^2$</td>
</tr>
<tr>
<td>Reported</td>
<td>10 (38.5)</td>
<td>3.65</td>
</tr>
<tr>
<td>Not reported</td>
<td>16 (61.5)</td>
<td>0.0559</td>
</tr>
<tr>
<td>Concentration disorder</td>
<td>Positive</td>
<td>$\chi^2$</td>
</tr>
<tr>
<td>Reported</td>
<td>5 (19.2)</td>
<td>0.49</td>
</tr>
<tr>
<td>Not reported</td>
<td>21 (80.8)</td>
<td>0.4829</td>
</tr>
<tr>
<td>Headache, bone, joint and muscle pain</td>
<td>Positive</td>
<td>$\chi^2$</td>
</tr>
<tr>
<td>Reported</td>
<td>10 (38.5)</td>
<td>5.21</td>
</tr>
<tr>
<td>Not reported</td>
<td>16 (61.5)</td>
<td>0.0225</td>
</tr>
</tbody>
</table>

*Significant diversity at $p<0.05$; Figures in parentheses indicate percentages.

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Table 2. \textit{Borrelia burgdorferi} and \textit{A. phagocytophilum} coinfection in relation to age, number of tick bite episodes, incidence of Erythema migrans and applied antibiotic therapy in Lyme borreliosis treatment

<table>
<thead>
<tr>
<th>Antibodies</th>
<th>Age (yr)</th>
<th>Number of tick bite episodes</th>
<th>Erythema migrans</th>
<th>Antibiotic therapy in Lyme borreliosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20–40</td>
<td>41–50</td>
<td>&gt; 50</td>
<td>Not reported</td>
</tr>
<tr>
<td>\textit{B. burgdorferi}</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>% Positive</td>
<td>21.9</td>
<td>29.6</td>
<td>32.4</td>
</tr>
<tr>
<td></td>
<td>% Negative</td>
<td>78.1</td>
<td>70.4</td>
<td>67.6</td>
</tr>
</tbody>
</table>

Test value: $\chi^2 = 0.95; p = 0.6214$; $\chi^2 = 0.64; p = 0.7276$; $\chi^2 = 0.80; p = 0.3708$; $\chi^2 = 0.72; p = 0.3964$. 

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<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Positive anti-\textit{B. burgdorferi}</th>
<th>Negative anti-\textit{A. phagocytophilum}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone and joint pain</td>
<td>Reported 19 (73.1)</td>
<td>46 (68.7)</td>
</tr>
<tr>
<td>Not reported</td>
<td>7 (26.9)</td>
<td>21 (31.3)</td>
</tr>
<tr>
<td>Muscle pain</td>
<td>Reported 16 (61.5)</td>
<td>32 (47.8)</td>
</tr>
<tr>
<td>Not reported</td>
<td>10 (38.5)</td>
<td>35 (52.2)</td>
</tr>
<tr>
<td>Headache</td>
<td>Reported 10 (38.5)</td>
<td>13 (19.4)</td>
</tr>
<tr>
<td>Not reported</td>
<td>16 (61.5)</td>
<td>54 (80.6)</td>
</tr>
<tr>
<td>Concentration disorder</td>
<td>Reported 5 (19.2)</td>
<td>9 (13.4)</td>
</tr>
<tr>
<td>Not reported</td>
<td>21 (80.8)</td>
<td>58 (86.6)</td>
</tr>
<tr>
<td>Headache, bone, joint and muscle pain</td>
<td>Reported 10 (38.5)</td>
<td>11 (16.4)</td>
</tr>
<tr>
<td>Not reported</td>
<td>16 (61.5)</td>
<td>56 (83.6)</td>
</tr>
</tbody>
</table>

*Significant diversity at $p<0.05$; Figures in parentheses indicate percentages.
However, a great variety of pathogens is transmitted by ticks, which may result in coinfections, in particular in subjects that are occupationally exposed to tick bites. The evaluation of tick infection is an important element in assessment of the risk of infection with tick-borne pathogens posed to humans. *Anaplasma phagocytophilum* and *B. microti* tick infections have been reported in different regions of Poland\(^{17-22}\). A study of *Ixodes ricinus* ticks in the northeastern part of Poland revealed that 8.7% of the tick population was infected with *A. phagocytophilum*. The percentage of the infected ticks was in the range of 2.3–13.7%, depending on the region\(^{20}\). The central-eastern part of Poland revealed that 8.7% of the tick population was infected with *A. phagocytophilum*. The presence of anti-*B. microti* antibodies was evaluated in two series of *B. microti*—1.05%; *B. burgdorferi* and *A. phagocytophilum*—0.93%, *B. burgdorferi* and *B. microti*—0.12%). There was only one co-infection which involved all the three pathogens (0.06%)\(^{22}\). The present study confirms the risk of infection with not only *B. burgdorferi*, but also *A. phagocytophilum* and *B. microti* in eastern Poland.

Walory et al\(^{10}\) examined healthy individuals bitten by ticks in the northeastern part of Poland. The presence of anti-*B. burgdorferi*, anti-*A. phagocytophilum* and anti-*B. microti* antibodies were evaluated in two series of samples from the same persons (interval 5–6 months). During the first collection, IgG anti-*B. burgdorferi* antibodies were found in 16% of individuals, IgG anti-*A. phagocytophilum* antibodies in 3.5%, and no positive results for IgG anti-*B. microti* antibodies were found. During the second collection, the antibodies against *B. burgdorferi* were found in 9.8% of individuals, against *A. phagocytophilum* in 4.9% and against *B. microti* in 1.4%. The first collection revealed one case of antibodies co-occurrence (anti-*B. burgdorferi* and anti-*A. phagocytophilum*) (0.7%). During the second collection these antibodies were detected in two patients (1.4%), while the presence of anti-*B. burgdorferi* and anti-*B. microti* antibodies was confirmed in only one case (0.7%).

The serum analyses of subjects suspected of Lyme borreliosis showed the presence of anti-*A. phagocytophilum* in 9.1% of the cases. Forestry work was the only statistically significant factor that increased the risk of the occurrence of antibodies\(^{20}\). Zwoliński et al\(^{23}\) reported a statistically significant difference (\(p<0.01\)) between the number of foresters  diagnosed with anti-*A. phagocytophilum* (19.8%) and the control group (5.4%). The co-occurrence of anti-*A. phagocytophilum* and anti-*B. burgdorferi* antibodies was detected in 4.5% of the foresters.

Grzeszczuk et al\(^{24}\) examined healthy adults employed in the Bialowiezki National Park (northeastern Poland) and found anti-*A. phagocytophilum* antibodies in 7.2% of the subjects, whereas co-occurrence of anti-*B. burgdorferi* and anti-*A. phagocytophilum* antibodies was found in 3.2%. Panczewicz et al\(^{25}\) examined foresters employed in the Bialowiezki National Park and found anti-*B. microti* antibodies in 9% of the subjects. The presence of anti-*B. burgdorferi* antibodies was detected in all the group members.

Studies carried out in the Lublin macroregion showed that 13.4% of ticks were infected with one of the three pathogens (*B. burgdorferi* 4.94%, *A. phagocytophilum* 4.94%, *B. microti* 3.52%), and 2.16% were coinfected (*A. phagocytophilum* and *B. microti*—1.05%; *B. burgdorferi* and *A. phagocytophilum*—0.93%, *B. burgdorferi* and *B. microti*—0.12%). There was only one co-infection which involved all the three pathogens (0.06%)\(^{22}\). The present study confirms the risk of infection with not only *B. burgdorferi*, but also *A. phagocytophilum* and *B. microti* in eastern Poland.

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The serum analyses of subjects suspected of Lyme borreliosis showed the presence of anti-*A. phagocytophilum* in 9.1% of the cases. Forestry work was the only statistically significant factor that increased the risk of the occurrence of antibodies\(^{20}\). Zwoliński et al\(^{23}\)
of coinfection with HGA varied from 2.3 to 10%, depending on the definition used.

In this study, coinfection with *A. phagocytophilum* was found in 28% of foresters and farmers diagnosed with the presence of IgG anti-*B. burgdorferi* antibodies. None of the studies in Poland cited above, demonstrated such a high proportion of subjects with anti-*A. phagocytophilum* antibodies. The results suggest a higher risk of *A. phagocytophilum* infection in the study area, in comparison with other regions of Poland. The analyses taking into account the age, sex, number of tick bite episodes, development of erythema migrans, and antibiotic therapy applied for Lyme borreliosis treatment revealed that the differences in the group diagnosed with *A. phagocytophilum* and *B. burgdorferi* coinfection were not statistically significant.

The percentage of subjects with the presence of anti-*B. microti* antibodies was low, i.e. 1.1% (only one person). This implies a low risk of infection with this pathogen after contact with ticks in this region. No case of simultaneous presence of antibodies against all the three pathogens (*B. burgdorferi*, *A. phagocytophilum*, and *B. microti*) was observed in this study.

The studies mentioned above confirm the coexistence of infections with different tick-borne pathogens. This may be of major importance for clinical procedures. Coinfection may give a non-specific clinical picture of the disease and thus hamper the diagnosis. The mechanisms of pathogen co-occurrence are not clear yet. It is believed that the *A. phagocytophilum* or *B. microti* coinfection may complicate the course of the disease in Lyme borreliosis patients. In our study, all the symptoms reported by the subjects (bone, joint, and muscle pain, headache, and concentration disorders) occurred more frequently among individuals diagnosed with *B. burgdorferi* and *A. phagocytophilum* coinfection, but the differences were not statistically significant. Remarkably, the more frequent headaches in the individuals coinfected with these pathogens were close to statistical significance. The statistical analysis indicated that the co-occurrence of headache, bone, joint and muscle pain was noted significantly more often among individuals diagnosed with *B. burgdorferi* and *A. phagocytophilum* coinfection.

*Anaplasma phagocytophilum* or *B. microti* coinfections might affect the clinical picture of other more common tick-borne diseases, especially Lyme disease caused by *Borrelia*. A correct diagnosis of mixed infections is not only important in terms of epidemiology, but essential to ensure appropriate treatment procedures in the fight against all the pathogens.

**CONCLUSION**

(1) Foresters and farmers are exposed to both mono- and coinfections with tick-borne pathogens. A high percentage of anti-*B. burgdorferi* and anti-*A. phagocytophilum* antibodies should prompt physicians to consider the probability of a coinfection with these pathogens in patients suspected of Lyme borreliosis. This should be taken into account when making diagnosis and decisions about the type of antibiotic therapy.

(2) The co-occurrence of headache plus bone, joint and muscle pain was noted significantly more often among individuals diagnosed with *B. burgdorferi* and *A. phagocytophilum* coinfection. Therefore, it is probable that these pathogens may severely interfere with the clinical course of Lyme borreliosis.

**Conflict of interest**

The authors declare that they have no conflict of interest.

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**REFERENCES**


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